REMARKS

Claims 1-14 are under examination, and Claims 15-43 have been withdrawn as non-elected subject matter.

1. Rejection of Claims Under 35 U.S.C. §103(a).

The Examiner has maintained the three rejections under 35 U.S.C. §103(a) made in the previous office action: (1) rejection of Claims 1-3 and 6 over Radin et al. in view of Lemons, Day et al. (US 6,358,531) and/or Inoue et al. (US 4,798,585); Claims 1-6 over Radin et al. in view of Lemons, and further in view of Day et al. (US 6,358,531) and/or Inoue et al. (US 4,798,585); and Claims 1-3 and 7-14 over Radin et al. in view of Lemons, Day et al. (US 6,358,531) and/or Inoue et al. (US 4,798,585) and further in view of Gerhart. To establish a *prima facie* case of obviousness under 35 U.S.C. §103(a), the Examiner must show that (1) the references teach all the elements of the claimed invention, (2) the references contain some teaching, suggestion or motivation to combine the references, and (3) the references suggest a reasonable expectation of success.

Initially, Applicants reiterate the arguments made in prior responses.

In all three rejections, the Examiner has cited Radin as disclosing "hollow calcium phosphate containing glass shells (abstract) that are combined with biologically active molecules such as BMP or collagen (bone mixture, tissues or by-products)(p.8)." Radin does disclose growth factors such as BMPs and cell attachment molecules such as collagen. Applicants submit, however, that such BMPs and collagen are not "bone mixture" as is claimed. As described on p. 10 of the specification, a "bone mixture" is a mixture containing bone which can be, for example, cancellous bone and/or demineralized bone matrix.

Further, in all three rejections, the Lemons, Day et al. and Inoue et al. references were cited as disclosing sintered calcium phosphate. However, the currently claimed materials do not recite that the calcium-containing microstructures are sintered. Rather, the current claimed subject matter is directed to a bone grafting composition comprising

(i) a bone graft extender comprising hollow calcium-containing microstructures and (ii) a bone mixture.

As further evidence of the patentability of the current claims, Applicants submit that the claimed compositions have unexpected effects. Attached hereto as Exhibit 1 is a report of experiments carried out on the subject matter of the invention, which demonstrate that when the composition in accordance with the present invention is used, containing hollow calcium-containing microstructures in an amount of 25% (the balance being demineralized bone matrix DMB), no significant difference is observed between the claimed composition and a control composition containing 100% DMB, in terms of viability as measured by the % of lacunae filled with osteocytes. (See Table 2, groups 4, 5, 7, and 8, compared to group 9 for size score). Even when the microstructures are substituted at a very high level (75% of the DMB), significant bone growth and viability were seen, as compared with a negative control of autoclaved DMB (Tables 2 and 3, groups 3 and 6 compared to group 10).

Accordingly, the present invention provides a bone graft extender (e.g., DMB which is costly and difficult to obtain), without significant loss of efficacy. The level of efficacy when the extender is used would not have been expected, since the claimed compositions represent a combination of a biologically inert material with a biologically active material.

Further, it has been found that, unexpectedly, the hollow calcium-containing microstructures of the present invention have improved crushing strength (measured as "aggregate modulus", that is , gradient of stress vs. strain graph for 20 microspheres in 2 cm^3 chamber loaded to strain levels 0.25, 0.50, 1.0, 1.5, and 2.0 at 0.4 mm/minute) compared with known calcium-containing microstructures. Exhibit 2 provides evidence of these results.

Applicants also note that the hollow microstructures provide improved bone growth compared with solid microstructures for a variety of reason, as explained on page 11 of the present application. Specifically, bone growth can occur within the hollow microstructure rather than around non-hollow particles, thereby providing a

better matrix for bone growth. Also, the hollow structure of the microstructures provides an optimal structure for maximizing bone in-growth and eventual replacement of the microstrucutures, while maintaining mechanical integrity of the implanted composition. The hollow material provides a lower mass of calcium containing material to be replaced at the implant site by host tissues. Moreover, hollow and solid microspheres can be compared in terms of available space for bone in-growth. For the hollow hydroxylapatite microspheres (HMD) listed in Exhibit 2 (diameter of 750 microns, shell thickness of 60 microns) and corresponding solid microspheres, a close-packed mass of either hollow or solid microspheres will have about 26% of its volume as interstitial space for bone in-growth. In addition, for hollow microspheres, the mass will have about 44% of its volume as internal space. Thus, a close packed mass of hollow microspheres will have about 70% of its volume available for bone in-growth, compared with about 26% for solid microspheres.

Based upon the foregoing, Applicants believe that all pending claims are in condition for allowance and such disposition is respectfully requested. In the event that a telephone conversation would further prosecution and/or expedite allowance, the Examiner is invited to contact the undersigned.

Respectfully submitted,

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Exhibit 1

Bone induction by hydroxyapatite beads with cancellous bone and bone matrix: A preliminary study in the rat

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Introduction

Bone defects created by neoplasms, cysts, trauma, infection and congenital defects can be surgically filled with a variety of substances. Goals include restoration of normal contour, restoration of mechanical strength and function, elimination of dead space to reduce postoperative infection and prevention of soft tissue ingrowth in order to encourage ingrowth of new bone. (1) Characteristics of an ideal substance used to fill bony defects are as follows: the material must be biodegradable, preventing areas of weakness or sites for possible infection after new bone is formed; porosity must be present to allow for early vascularity and bone growth development; the substance must be completely biocompatible without an inflammatory reaction; the material must be sterilizable without a change in its properties, and the material must be readily available and at a low cost.

Small defects can be filled with cancellous bone grafts. (1) This is useful, but is limited by the amount of cancellous bone which can be harvested in an individual, and by donor site morbidity. Large defects can be replaced by cortical allografts requiring the use of donors or a bone bank. Along with this are concerns over infectious disease transmission from donor to recipient patients.

Subchondral bone defects are generally treated with curretage, followed by cancellous bone replacement. (2) Lack of support for articular cartilage can result in joint collapse. Methyl methacrylate has been used for bone replacement, but concerns exist over its biomechanical properties, in that it is too stiff, resulting in degenerative changes in adjacent articular cartilage. It also may have thermal and toxic side effects on adjacent tissues. The reported advantage is rapid return to full weight bearing.

A variety of natural and synthetic compounds have been investigated for implantation into cortical defects. Calcium sulfate has been successfully implanted in cortical defects of long bones in dogs (1,3,4), maxillary dentition extraction defects in humans (5), and unicameral bone cyst defects in humans (6). It acts as a filling substance to allow normal bone healing, but has no osteogenic properties. Calcium sulfate has also been evaluated as a carrier for sustained release of antibiotics. (7)

Synthetic hydroxyapatite (HA, $Ca_{10}[PO_4]_6[OH]_2$) as well as other calcium phosphate substances, have been evaluated for use in filling bone defects since their structure mimics that of the crystalline structure of bone. Studies have established equivalent to superior strength of HA, compared to bone grafts, both when tested ex vivo and when placed in bone defect models. (8-11). Hydroxyapatite has been evaluated as an adjunct to repair of stripped cancellous screw holes and unstable fractures. (12,13) Biocompatibility, minimal inflammatory response and equivalent new bone formation over time, compared to defects filled with DMB, have been demonstrated for HA. (8,14). Hydroxyapatite has also been used as a carrier for the sustained release of antimicrobials. (15, 16) Controlled studies evaluating the effect of hydroxyapatite on bone induction have not been performed.

The present, clinical use of bone fillers, including HA beads, usually involves combining either demineralized bone matrix (DMB) or cancellous bone graft with the filling compound to assist in bone induction. A pre-made product of HA, DMB and/or cancellous bone would combine the structural advantages of HA with the advantages of bone induction from the DMB and cancellous bone.

This study was directed at characterizing the chronology of bone ingrowth of various bone replacements in muscle pouches in nude athymic rats; comparing that of dense and porous

HA beads alone or in combination with cancellous bone and DMB with positive (sterilely harvested DMB) and negative (autoclaved DMB) controls.

Materials and Methods

Implant Preparation:

Sterilely harvested human bone was processed, using established protocols, into demineralized bone matrix powder (DMB) and cancellous bone (CAN) at Allosource, Denver CO. Calcium concentrations were evaluated on a dry weight basis. Both dense and porous HA beads were prepared using an established protocol, also at Allosource. Negative and positive controls were aseptically prepared by combining 10 mg of bone matrix with 0.05 ml of phosphate buffered saline to form a paste. The paste was placed into the end of a 1-ml syringe with the luer lock removed. This formed a delivery mechanism for the DBM pellet. Combinations of DMB, cancellous bone and HA beads were created for 20 replicates in the groups listed in Table 1.

Surgical implantation:

Fifty athymic nude rats (Tac:N:NIH-rnuDF) were individually housed in separate isolators (Thorn Co., Hazelton PA) in a room equipped with laminar flow. The rats were fed and watered using an irradiated pellet ration and irradiated water. Sterile shavings were used for bedding. The animals were cared for in an accredited laboratory animal facility and the protocol approved by the Colorado State University Animal Care and Use Committee. Anesthesia was induced and maintained using isofluorane gas by face mask. The hair on the dorsum was clipped from the base of the skull to the caudal lumbar region. The shaved region was aseptically prepared using an antiseptic solution (Hibicleans R, Stuart Pharmaceuticals, Wilmington, Delaware) alternating with 70% isopropyl alcohol. Following sterile draping, a 4 cm skin incision was made on

midline. Sharp and blunt dissection was used to expose the epaxial musculature. Four muscle pouches were created. Sharp dissection was performed to produce a muscle pouch one cm long and 0.5 cm deep into the epaxial musculature. Sample groups were assigned to muscle pouch sites in a rotating pattern; to result in 20 replicates of each group. A single simple interrupted suture of 4-0 polyglyconate (Maxon^{R}, Davis & Geck) was used to close the muscle pouch. Skin closure was performed using a continuous intradermal suture of 4-0 polyglyconate. The rats were allowed to recover in their individual isolators and observed daily for any complications to the surgical procedure. Hydrocodone was added to the water of all rats for a duration of three days for alleviation of postoperative pain.

Evaluation of Bone Induction:

Euthanasia was performed, using a 70% CO_2 chamber, on one half of the rats (equivalent to 10 replicate sites for each group) once positive control sites demonstrate palpable bone growth. The remaining rats were euthanized at 12 weeks post implantation. Each implantation site was dissected free, maintaining 0.5 cm of normal epaxial muscle tissue around the site. The tissue was placed into individual cassettes and then into 10% neutral buffered formalin for three days. The cassettes were then placed in 10% formic acid with ion exchange resin for 48 hours for decalcification, then processed in paraffin and sectioned in 5 micron sections, placed on slides and stained with hematoxalin and eosin. Slides were evaluated and scored as in Table 2.Viability and size scores were compared between groups as well as within groups and between sacrifice times using a one-way analysis of variance (ANOVA) using a statistical software package (SPSS 10.1, Chicago, Illinois). Significance was determined at a p-value of less than or equal to 0.05.

Results

Tables 2 and 3 list the average viability scores for each group following the 9 and 18 week sacrifice. Table 3 lists the average size score for each group following the 18 week sacrifice. The viability and size scores for the negative control group as well as for both dense and porous bead (only) groups were zero. Consistently, fibrous tissue ingrowth was seen surrounding the HA beads. A significant difference was found only for group 3 (DB + 25 mg DMB, $p = 0.019$) and group 6 (PB + 25 mg DMB, $p = 0.007$) when comparing between the 2 sacrifice times. No difference was seen between sacrifice times for the positive or negative control groups (9 & 10, respectively). For viability score, a significant difference was found between groups containing increasing amounts of DMB and those containing less (75 mg vs 50 mg vs 25 mg) for both dense ($p < 0.001$) and porous beads ($p < 0.001$). For size score, a significant difference was found between group 5 (DB + 75 mg DMB) and group 3 (DB + 25 mg DMB), favoring group 5 ($p = 0.008$), but not between group 5 and group 4 (DB + 50 mg DMB, $p = 0.15$)). A significant size difference was found between groups containing increasing amounts of DMB and those containing less (75 mg vs 50 mg vs 25 mg, $p = 0.045$) for the porous bead groups (8,7 & 6, respectively). All dense and porous bead groups containing DMB had significantly greater viability and size scores compared to the negative control group (group 10) and the bead only groups (groups 1 & 2). No difference was found between dense and porous bead groups containing the same amount of DMB. The positive control group (group 9) had significantly greater viability scores compared to both dense and porous beads containing 25 mg or 50 mg DMB (groups 3,4,6 & 7), however, no significant difference was found between positive control and dense or porous bead groups containing 75 mg DMB (groups 5 & 8). A significant difference for size scores was only seen between the positive control groups and dense and porous bead groups containing 25 mg DMB (groups 3 ($p = 0.018$) and group 6 ($p = 0.007$)). No

difference was seen between positive control groups and dense or porous bead groups containing 50 mg or 75 mg DMB (groups 4,5,7 & 8).

Discussion

This study supports this in vivo model of osteoinduction as all rat sites from the positive control group (group 9) had at least a viability score of 1 and a size score of 3. This compared to the negative control group (group 10) which had no sites with positive viability scores and only on site with a size score of 1; the remainder being zero. These results would compare to previous studies using this model.(17, Ferreira SD, Dernell WS, Powers BE, et al. Effects of gas plasma sterilization on demineralized bone matrix. Clin Orthop Rel Res. (In Press)). This study indicates that HA beads alone do not appear to possess osteogenic properties, as beads alone did not result in any bone formation. This is expected since no osteoinductive elements are present within the beads and their indicated use is for structural support and possibly osteoconduction. (18, 19) The combination of HA beads and DMB does result in osteoinduction, with increased bone production as the amount of DMB is increased. There does not appear to be a difference between the dense or porous beads on the osteoinduction of the DMB. Both dense and porous HA beads may, however, suppress or at least delay osteogenesis and osteoinduction when combined with CAN and DMB. This is evident at both time points as the beads combined with DMB never surpassed the bone growth induced by the positive controls, with groups 5 and 8 having 7.5 times as much DMB implanted. Bone was not observed to grow in close association with HA beads of either type, rather there appeared to be fibrous tissue formation surrounding the beads. This same reaction has been seen in previous studies. (8,14) This reaction may have inhibited bone formation, at least in close proximity to the bead. This could be due to the manner of

implantation as a separation often occurred between the bead and the CAN/DMB. This might be compensated for if the beads and DMB were delivered in a manner that ensured their close association once implanted. It is possible that the HA beads merely delayed osteoinduction, rather than truly inhibiting it. Both groups 3 and 6 showed significant differences for viability scores between the two sacrifice times, favoring the later sacrifice. Although this was not true for any of the other bead groups containing DMB, it is possible that a later sacrifice may result in increased bone production, lessening the differences between bead groups and the positive control.

Conclusions

This study supports the use of this *in vivo* model of osteoinduction. It appears that HA beads alone do not result in osteoinduction. However, when combined with DMB, osteoinduction does occur; the degree of which is related to the amount of DMB. Hydroxyapatite beads combined with DMB resulted in less bone formation than positive control groups when comparing equal amounts of DMB. This may be the result of inhibition or delay resulting from the presence of the bead; however, further evaluation is needed to elucidate this relationship. It appears from this work that the combination of HA beads and CAN/DMB may offer a viable treatment option for the replacement of bone defects.

Table 1. Treatment group composition

Group Number	Implanted material
1	Dense Beads (DB)
2	Porous Beads (PB)
3	DB with CAN and 25 mg DMB
4	DB with CAN and 50 mg DMB
5	DB with CAN and 75 mg DMB
6	PB with CAN and 25 mg DMB
7	PB with CAN and 50 mg DMB
8	PB with CAN and 75 mg DMB
9	10 mg DMB alone (positive control)
10	Autoclaved 10 mg DMB (negative control)

Table 2. Viability and size scores

Viability Score: 1=<10% of lacunae filled with osteocytes

2=10-25% of lacunae filled with osteocytes

3=>25% of lacunae filled with osteocytes

Size Score (for 2nd sacrifice only)= number of 10x fields that the bone occupies

Table 2. Average viability scores for each of the 10 implant groups following sacrifice at 9 weeks following implantation

Group	Average Viability Score	Std Dev
1	0.00	0.00
2	0.00	0.00
3	0.27	0.65
4	0.82	1.08
5	1.60	1.35
6	0.20	0.42
7	0.90	1.29
8	1.70	1.06
9	2.40	0.84
10	0.00	0.00

Table 3. Average viability and size scores for each of the implant groups at 18 weeks following implantation

Group	Average Viability Score	Std Dev	Average Size Score	Std Dev
1	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00
3	0.89	0.60	2.44	1.74
4	1.33	1.00	3.56	1.67
5	2.00	0.87	5.11	1.96
6	1.33	1.22	2.89	2.37
7	1.60	1.17	4.00	1.25
8	2.40	0.70	6.50	2.68
9	2.44	0.73	5.22	1.92
10	0.00	0.00	0.00	0.00

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Exhibit 2

Aggregate Moduli Comparison

The aggregate moduli (H_A)of two compositions of hollow microspheres (HMD and BMP) were compared with those of other commercial and bone implant materials. None of the materials evaluated, except for the test materials (HMD and BMP), were hollow. 2 cc samples of each material were evaluated and the results are shown below in Table 1.

Material (no. of samples)	HA (mean ± stnd dev)
Hollow hydroxylapatite microspheres (HMD)--~750um diameter, ~60 um shell thickness (2)	109.7 ± 0.30 Mpa
Perioglas®—solid non-porous particulate synthetic material (NovaBone Products, LLC, Alachua, FL, USA) (3)	29.2 ± 0.11 Mpa*
Cortical Bone Chips (3)	23.3 ± 0.06 Mpa*
Hollow tricalcium phosphate microspheres (BMP) (2)	18.5 ± 0.14 Mpa*
Bio-Oss®--natural bone substitute material (hydroxylapatite) obtained from the mineral portion of bovine bone (Geistlich Biomaterials, Inc., Wolhusen, Switzerland) (3)	6.61 ± 0.14 Mpa*/**
Pro Osteon®--harvested from marine coral exoskeletons that are hydrothermally converted to hydroxylapatite. 500 um pore size (Interpore Cross International, Irvine, CA, USA) (2)	5.02 ± 0.03 Mpa*/**
Cancellous bone (3)	1.72 ± 0.01 Mpa*/**
*Significantly different from HMD $p \leq 0.05$ **Significantly different from BMP $p \leq 0.05$	